

# Site-Directed Integration of Large DNA Sequences into Endogenous Sites in the Human Genome using an engineered Modular Integrase (MINT) Platform

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FASEB Genome Engineering June 17, 2024



## Disclosures

I am a full-time employee of Sangamo Therapeutics, Inc.

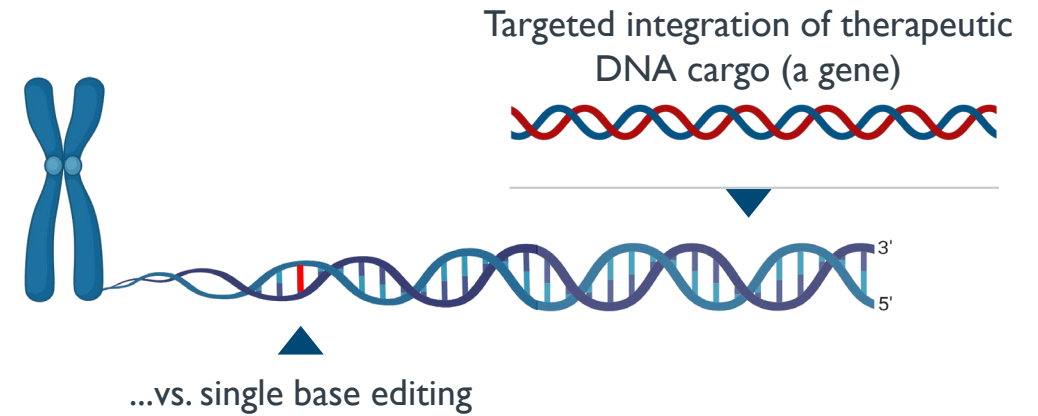
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# Integrases Will Write the Next Chapter of Genomic Medicine

## Integrases meet the requirements for ideal therapeutic agents

- ✓ Capable of delivering large payloads - 10 kb+
- ✓ No copying required - low error rate
- ✓ Self sufficient - no dependence on cell DNA repair machinery
- ✓ No DNA breaks - reduced translocation risk



*“if it was possible to integrate very large DNA sequences where you could integrate entire genes or sets of genes into a targeted position in the genome [it] would be very powerful” –Jennifer Doudna*

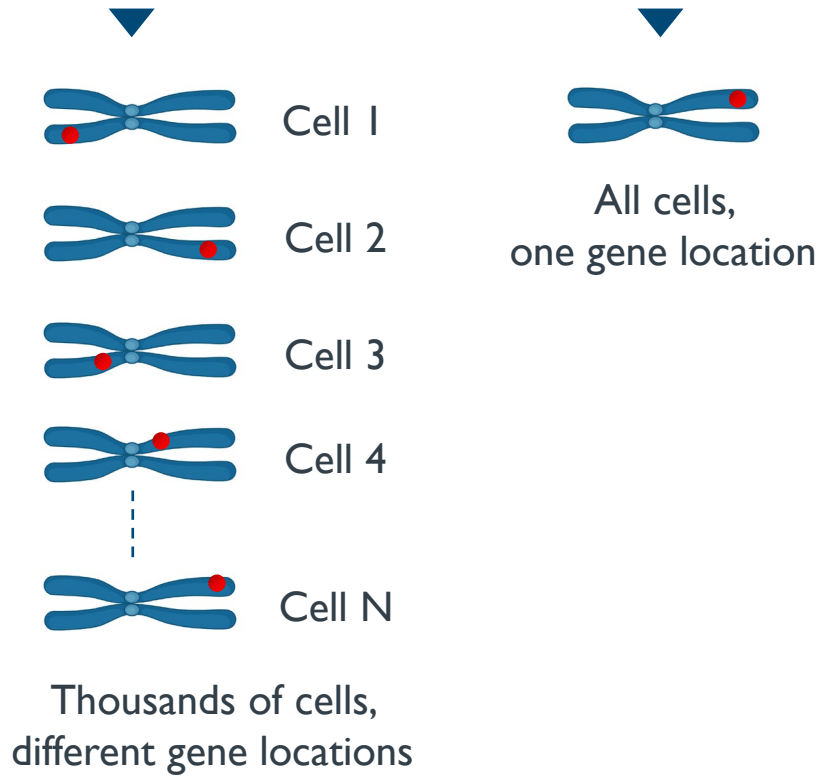
*“a hypothetical fully programmable recombinase would be in some respects an ultimate genome editing agent” –David Liu*

**CRISPR roundtable**

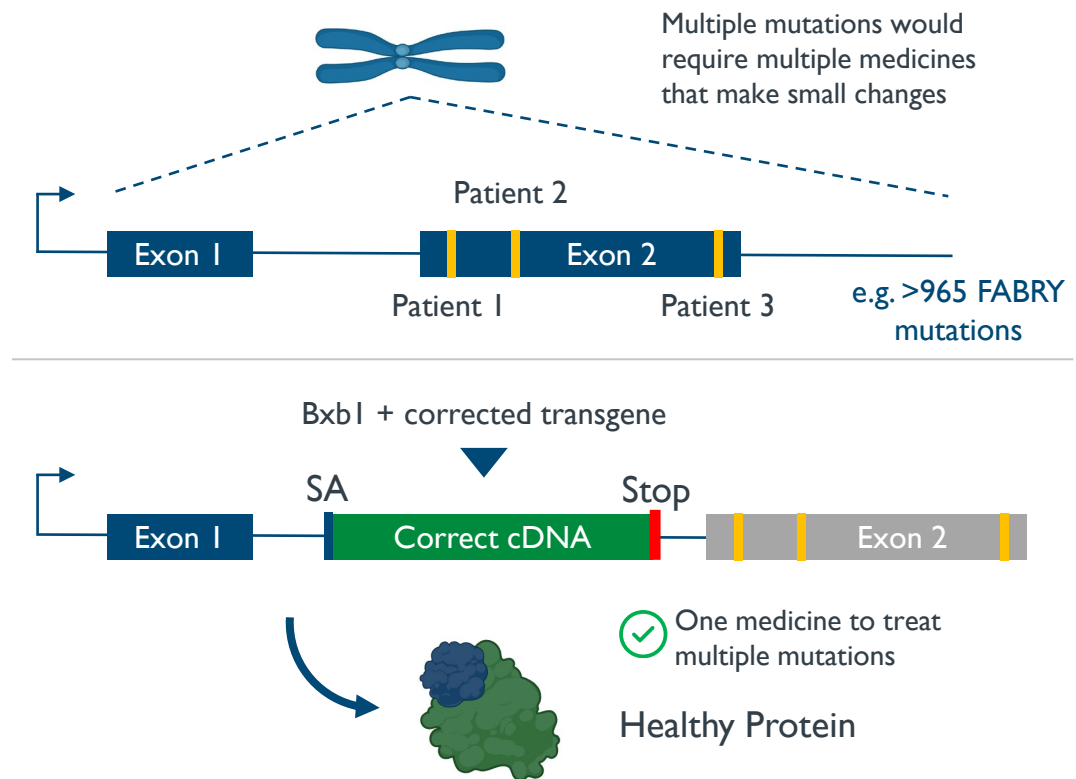


# Targeted Integration Improves Existing Therapies and Enables New Therapies

## Random vs. Targeted Random Integration



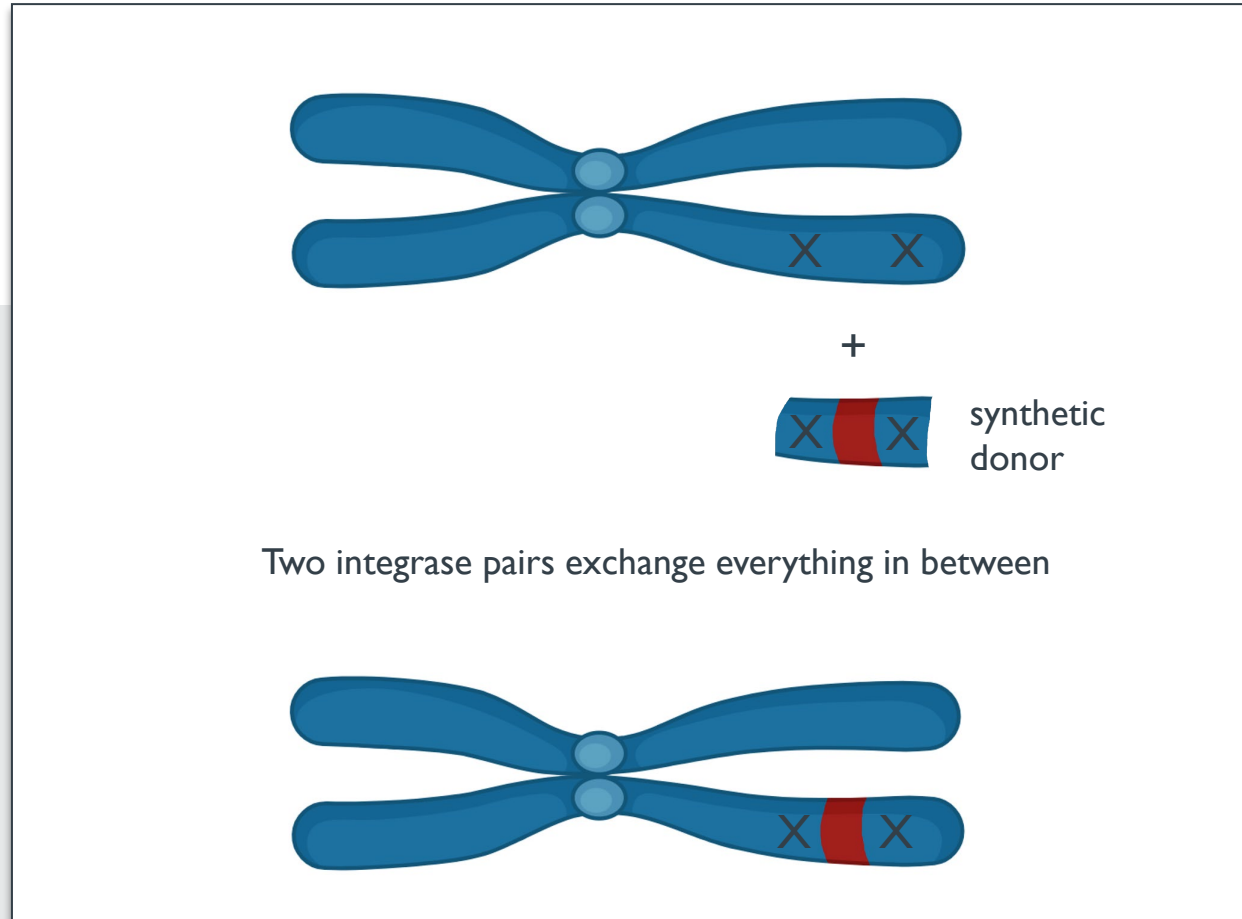
## One medicine to treat all mutations



Images by Biorender



# Towards Cassette Exchange - the Ultimate Genome Engineering Tool



Ideal for simultaneously changing multiple genes

Aspirational example: replace large regions of a chromosome such as the entire MHC locus to avoid transplant rejection

Images by Biorender



# Retargeted Recombinases are a Long-standing Challenge for the Field

2003

## Chimeric recombinases with designed DNA sequence recognition

Aram Akopian, Jiuya He\*, Martin R. Boocock, and W. Marshall Stark†

Published online 26 September 2012

*Nucleic Acids Research*, 2012, Vol. 40, No. 21 11163–11172  
doi:10.1093/nar/gks875

## Chimeric TALE recombinases with programmable DNA sequence specificity

Andrew C. Mercer, Thomas Gaj, Roberta P. Fuller and Carlos F. Barbas III\*

2012

2007

## Evolution of Programmable Zinc Finger-recombinases with Activity in Human Cells

Russell M. Gordley, Justin D. Smith, Torbjörn Gräslund and Carlos F. Barbas III \*



9758–9770 *Nucleic Acids Research*, 2016, Vol. 44, No. 20  
doi: 10.1093/nar/gkw707

Published online 11 August 2016

## A programmable Cas9-serine recombinase fusion protein that operates on DNA sequences in mammalian cells

Brian Chaikind<sup>1,2</sup>, Jeffrey L. Bessen<sup>1,2</sup>, David B. Thompson<sup>1,3</sup>, Johnny H. Hu<sup>1,3</sup> and David R. Liu<sup>1,2,\*</sup>

2016

2010

## Directed evolution of recombinase specificity by split gene reassembly

Charles A. Gersbach, Thomas Gaj, Russell M. Gordley and Carlos F. Barbas III\*

4198–4206 *Nucleic Acids Research*, 2010, Vol. 38, No. 12  
doi:10.1093/nar/gkq125

nature  
biotechnology

ARTICLES

## Directed evolution of a recombinase that excises the provirus of most HIV-1 primary isolates with high specificity

Janet Karpinski<sup>1,2,11</sup>, Ilona Hauber<sup>2,11</sup>, Jan Chemnitz<sup>2,11</sup>, Carola Schäfer<sup>2,3</sup>, Maciej Paszkowski-Rogacz<sup>1</sup>, Debayoti Chakraborty<sup>1</sup>, Niklas Beschorner<sup>2</sup>, Helga Hofmann-Sieber<sup>2,3</sup>, Ulrike C Lange<sup>2-4</sup>, Adam Grundhoff<sup>2,3</sup>, Karl Hackmann<sup>5</sup>, Evelin Schrock<sup>5</sup>, Josephine Abi-Ghanem<sup>6</sup>, M Teresa Pisabarro<sup>6</sup>, Vineeth Surendranath<sup>7</sup>, Axel Schambach<sup>8</sup>, Christoph Lindner<sup>9</sup>, Jan van Lunzen<sup>2,3,10</sup>, Joachim Hauber<sup>2,3</sup> & Frank Buchholz<sup>1,7</sup>

2016

2011

## Structure-guided reprogramming of serine recombinase DNA sequence specificity

Thomas Gaj, Andrew C. Mercer, Charles A. Gersbach<sup>2</sup>, Russell M. Gordley, and Carlos F. Barbas III<sup>1</sup>

nature biotechnology



Article

<https://doi.org/10.1038/s41587-023-02121-y>

## Activation of recombinases at specific DNA loci by zinc-finger domain insertions

2024

Received: 18 April 2023

Accepted: 22 December 2023

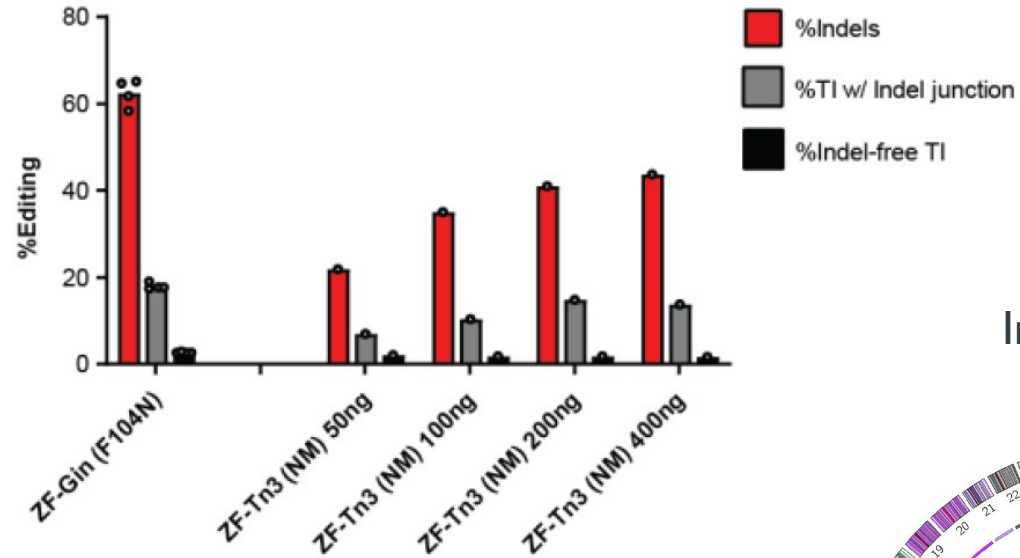
Published online: 31 January 2024

Liliya Mukhametzyanova<sup>1</sup>, Lukas Theo Schmitt<sup>1,2</sup>, Julia Torres-Rivera<sup>1</sup>, Teresa Rojo-Romanos<sup>1,2</sup>, Felix Lansing<sup>1,2</sup>, Maciej Paszkowski-Rogacz<sup>1</sup>, Heike Hollak<sup>1,2</sup>, Melanie Brux<sup>1</sup>, Martina Augsburg<sup>1</sup>, Paul Martin Schneider<sup>1,2</sup> & Frank Buchholz<sup>1,2</sup>✉

# Recombinase Catalytic Domain Fusions can Result in Product Purity Issues

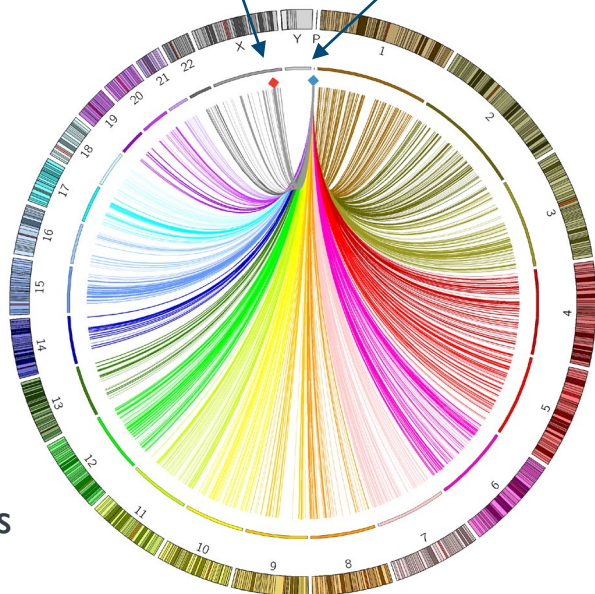
Taking the recombinase catalytic domain out of its native context is the likely cause of these issues

Editing in Human K562 cells



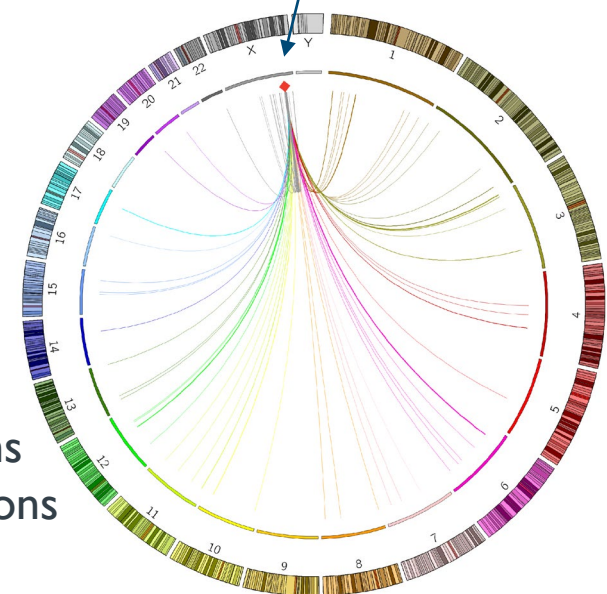
Intended target

Donor



ZF-Gin  
Genome-wide  
integrations

Intended target



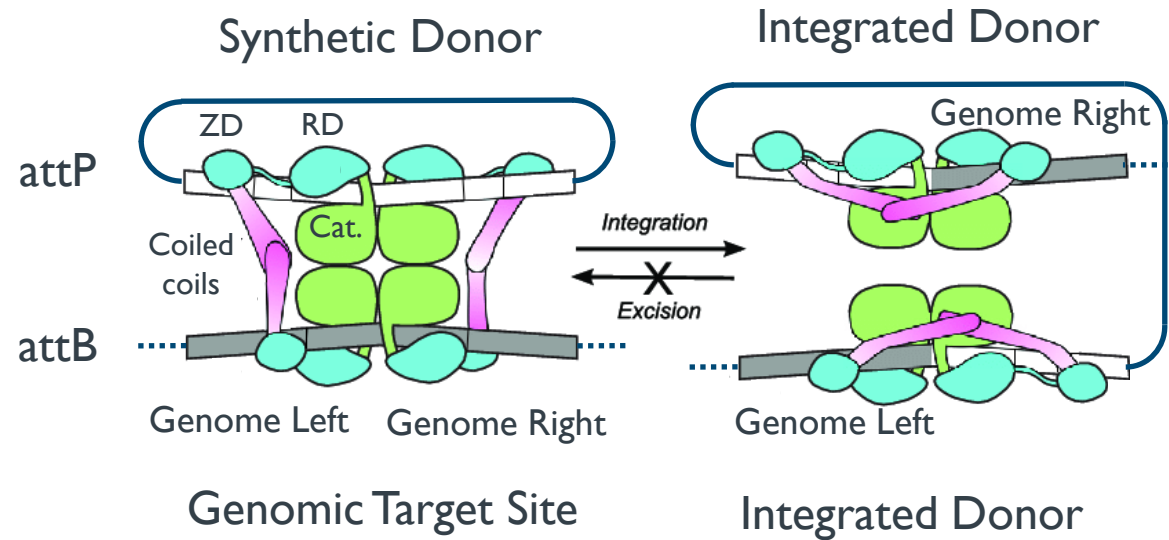
ZF-Gin  
Translocations  
& Large Deletions



Jessica Davis



# Reprogrammed Serine Integrases Would be Ideal for Therapeutic Applications

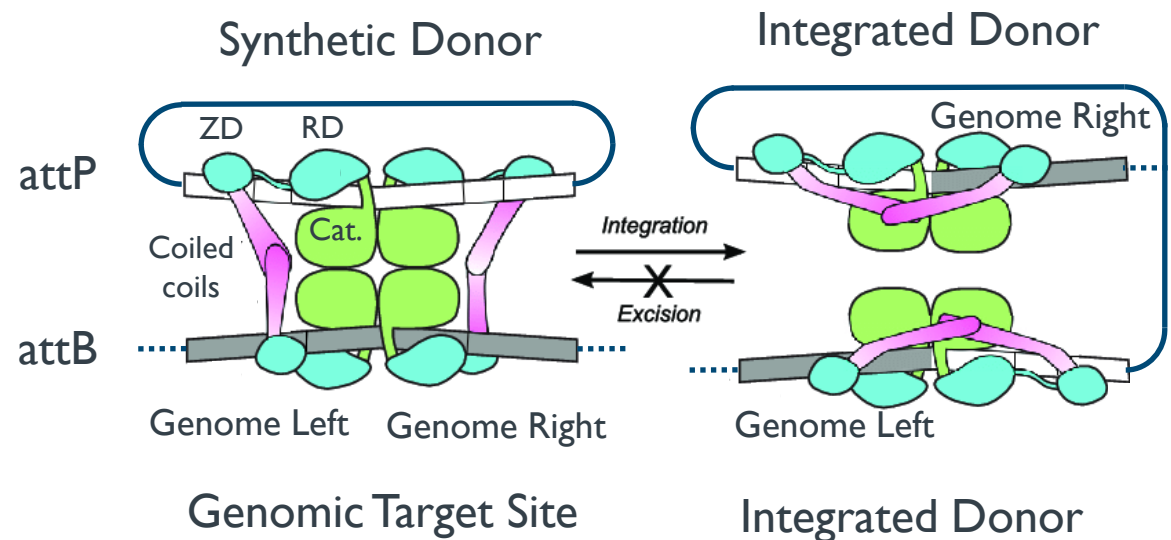


Adapted from Gupta et al., NAR (2017)

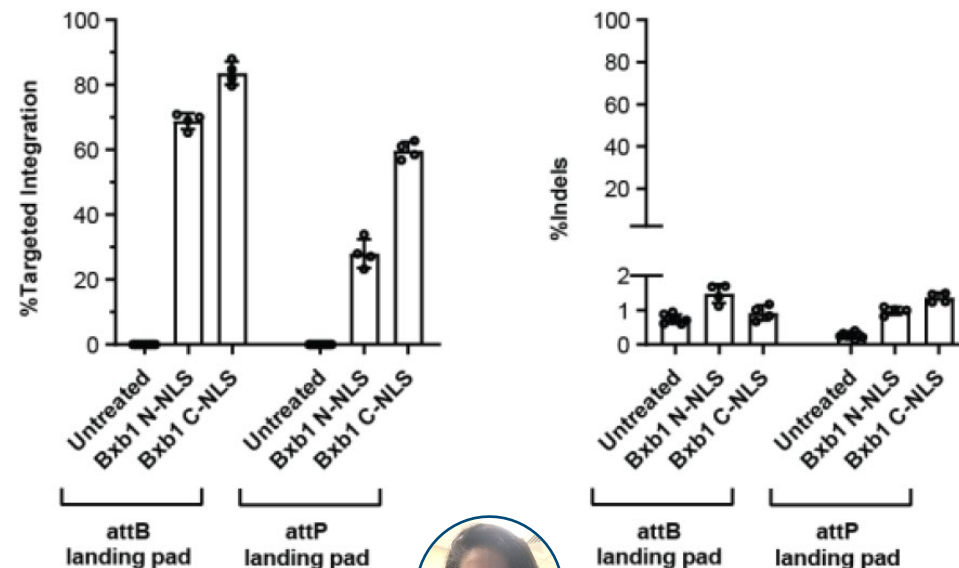
- **Irreversible integration:** Integrase coiled-coil domains ensure integration is unidirectional
- **Large cargo delivery:** Native enzyme integrates 50 kb cargo into host genome
- **2 bp of specificity from donor:** Central dinucleotides of attP and attB must match for integration
- **Components compatible with therapeutic delivery:** enzyme is ~500 residues & no co-factors



# Reprogrammed Serine Integrases Would be Ideal for Therapeutic Applications



Adapted from Gupta et al., NAR (2017)



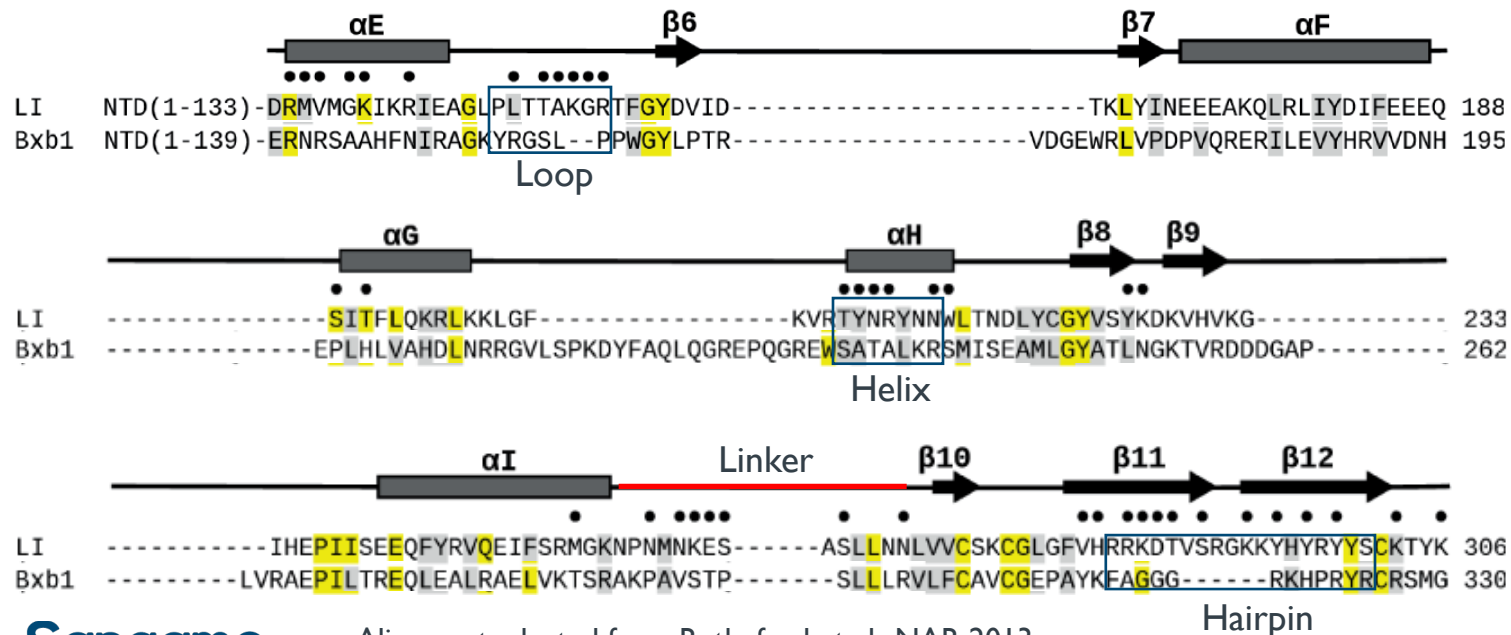
Bhakti Kadam

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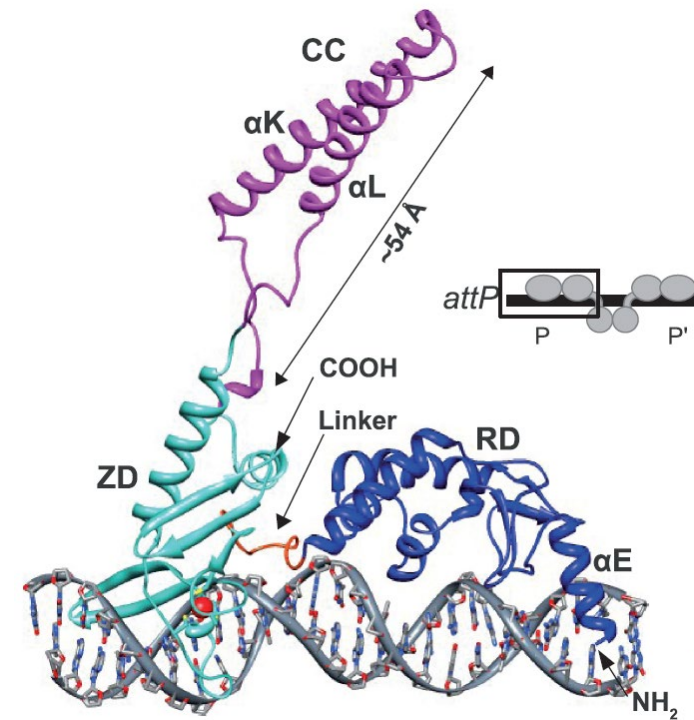


# Challenges of Directly Reprogramming Bxb1 (i.e. why hasn't anyone else done this yet?)

- No “simple code” for Bxb1 reprogramming
- Replacing DNA recognition domain with ZFs, TALEs, or CRISPRs will break control machinery
- Will require re-engineering the protein-DNA interface (that's what we do!)
- No existing 3D crystal structure for Bxb1
- Recent AI-methods don't dock Bxb1 model with DNA correctly
- Most relevant structure is of distantly related LI integrase



Alignment adapted from Rutheford et al., NAR 2013

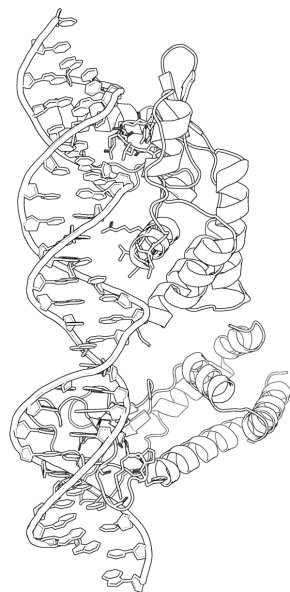


LI integrase structure – 4KIS.pdb



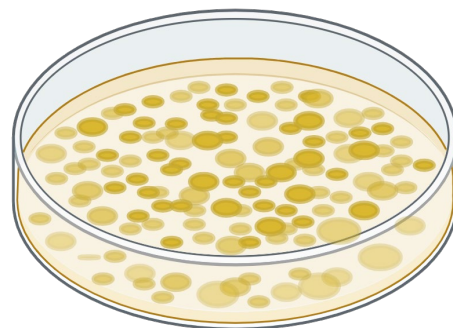
# Our Integrase Reprogramming Strategy

## Model



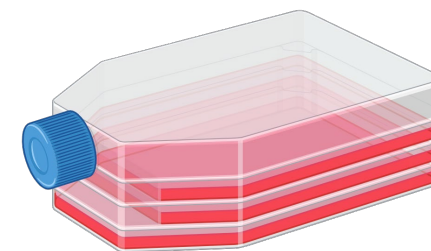
- AI-driven 3D Structural modeling
- Experimental Mapping of DNA-protein interactions

## Directed evolution



- Bacterial selection
- Up to 1 billion mutants

## Test and validate

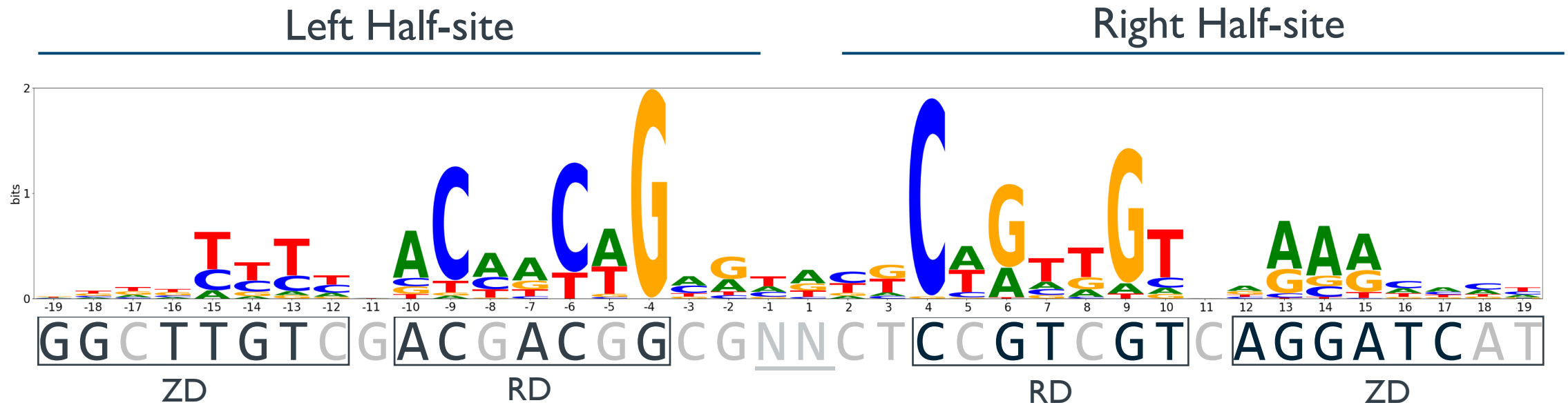


- Activity in human cells
- Start with sites where wt Bxb1 has weak, but detectable activity



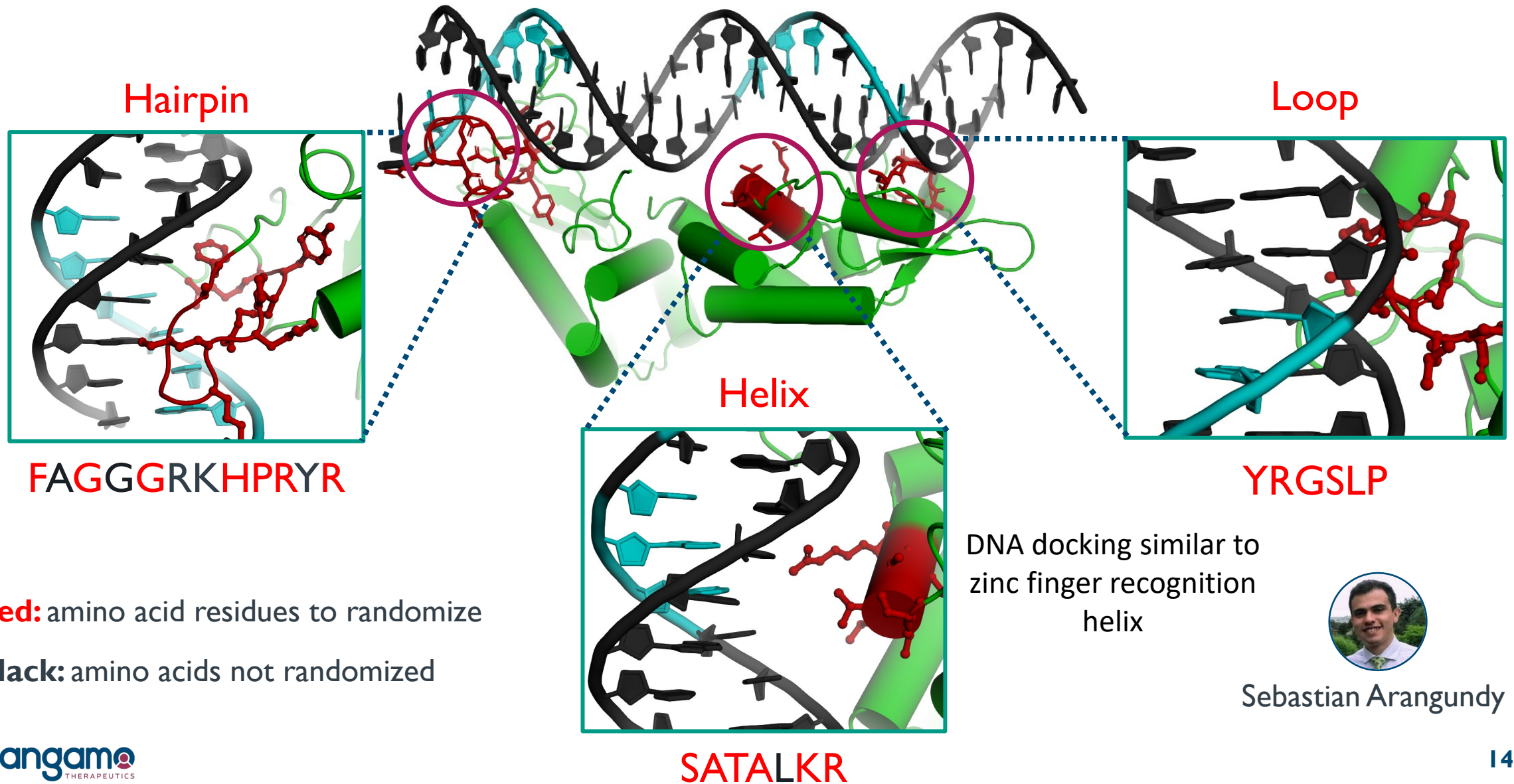
# Bxb1 Target Sites within the Human Genome

- Computational search based on data from Bessen *et al.* identified 8 attB-like sites with at least 0.1% TI, but no active attP-like sites identified
- Genome-wide integration assay in K562 cells using a mixture of donor constructs with all possible central dinucleotide sequences identified 89 attB-like sites, but no attP-like sites
- 15 experimentally identified attB-like sites validated with at least 0.1% TI and 12 of 15 are in genes
- Sequence logo of 89 experimentally identified sites is consistent with natural attB site





# Structural Model and Mapped Interactions Suggest Bxb1 Engineering Strategy





# Bacterial Selections can Reprogram the Bxb1 Helix

~1 billion variants

P T D V L P K  
 A W R I L S K  
 Y G H K L A I  
 Y H L R L P S  
 W T A W L G S  
 R R T M L S P  
 V M L L L V W  
 V V V Q L A R  
 I P L S L L T  
 I W P P L M G  
 L L N V L I P  
 Y M N C L S W  
 P H R V L A A  
 E T C S L L H  
 P Y V A L K W  
 T Q R P L S L  
 P A G P L K P  
 R R T G L K A  
 T K P G L A R  
 L R A H L C L  
 V W E A L P G  
 A T A S L T H  
 S H V K L G R  
 R K Q L L G P  
 A M I C L A P  
 L L V R L E E  
 I S W G L N K  
 A G T V L R T  
 N A L K L G L  
 R F S L L F R  
 M L W C L M A  
 D T R D L S Q  
 E E R Q L G L  
 D D L R L R R  
 T P G T L L P

Chaos

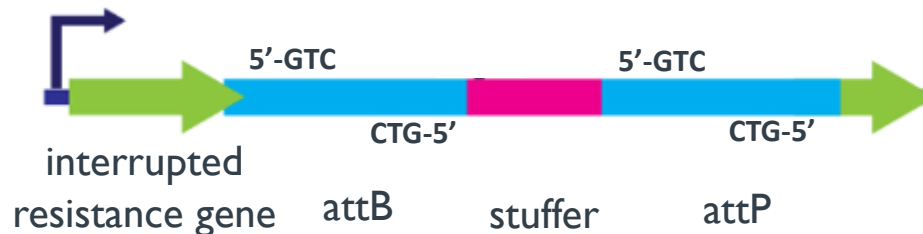
Bxb I Variant Library



antibiotic selection (single round)



Target Construct



~100 candidate integrases

R P G N L K R  
 R G T S L K R  
 H A G S L K R  
 A P Q N L K R  
 Y P T S L R R  
 H T T S L K R  
 R S G N L R R  
 Y A T S L K R  
 R A G N L R R  
 T G G N L R R  
 R G N N L R R  
 W A S S L R R  
 A G N N L R R  
 R S N N L K R  
 H A T S L K R  
 H A Q N L R R  
 W A T S L R R  
 R G G N L K R  
 W S Q N L K R  
 R G H N L K R  
 Y G S N L K R  
 R G N S L K R  
 Y G N N L K R  
 W S G S L R R  
 W P G S L K R  
 W A S T L K R  
 H Q G S L K R  
 R S S S L K R  
 Y A S T L K R  
 H A R N L K R  
 W P S T L K R  
 F S T S L K R  
 T S G N L K R

Order

Bxb I natural half-site

GGCTTGTGACGGCG  
 ATGCTCCTGTCGGCG

Human DNA target half-site identified computationally



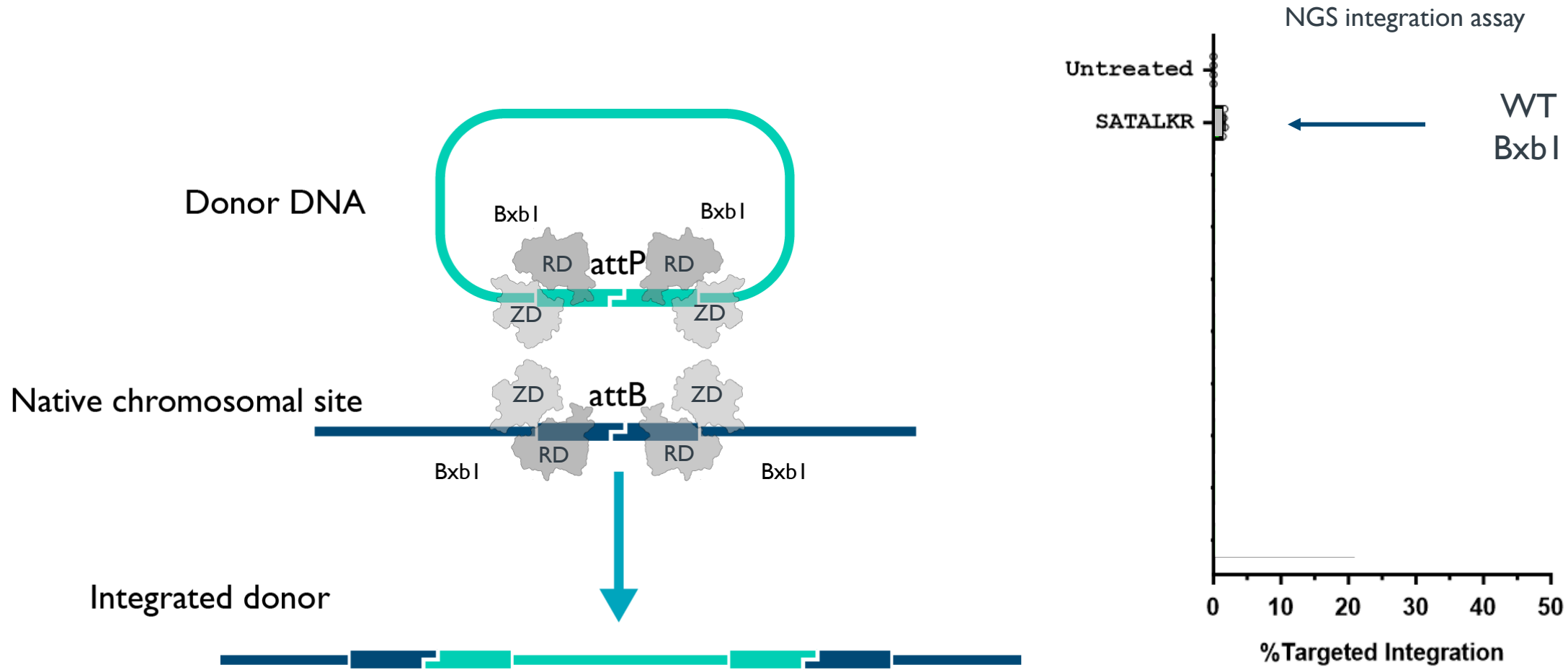
Sebastian Arangundy



Luis Rodriguez



# Selected Bxb1 Helices Enable Integration into the Genome of Human Cells



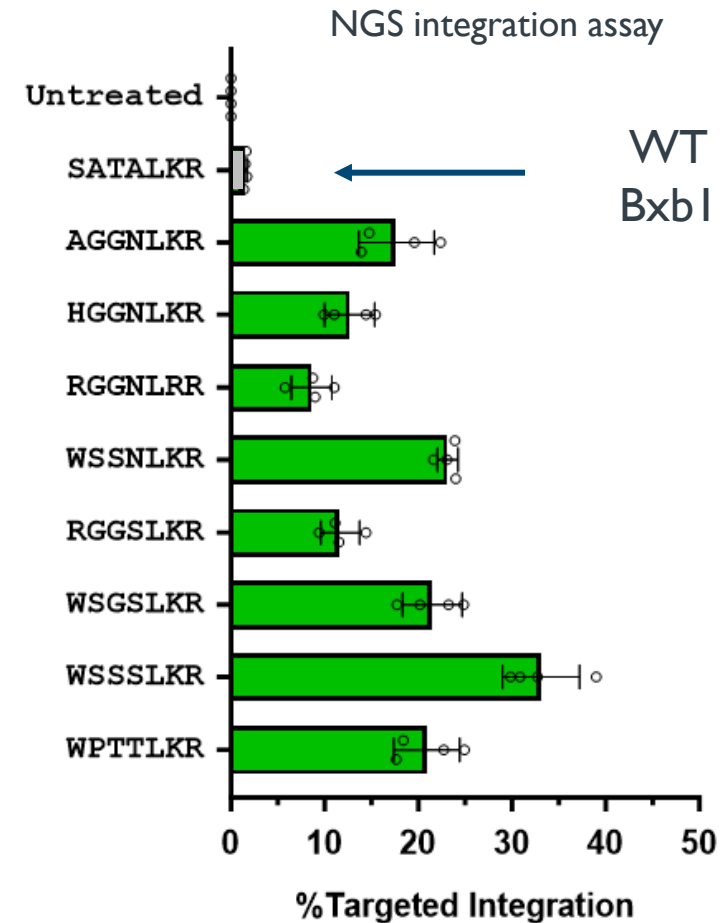
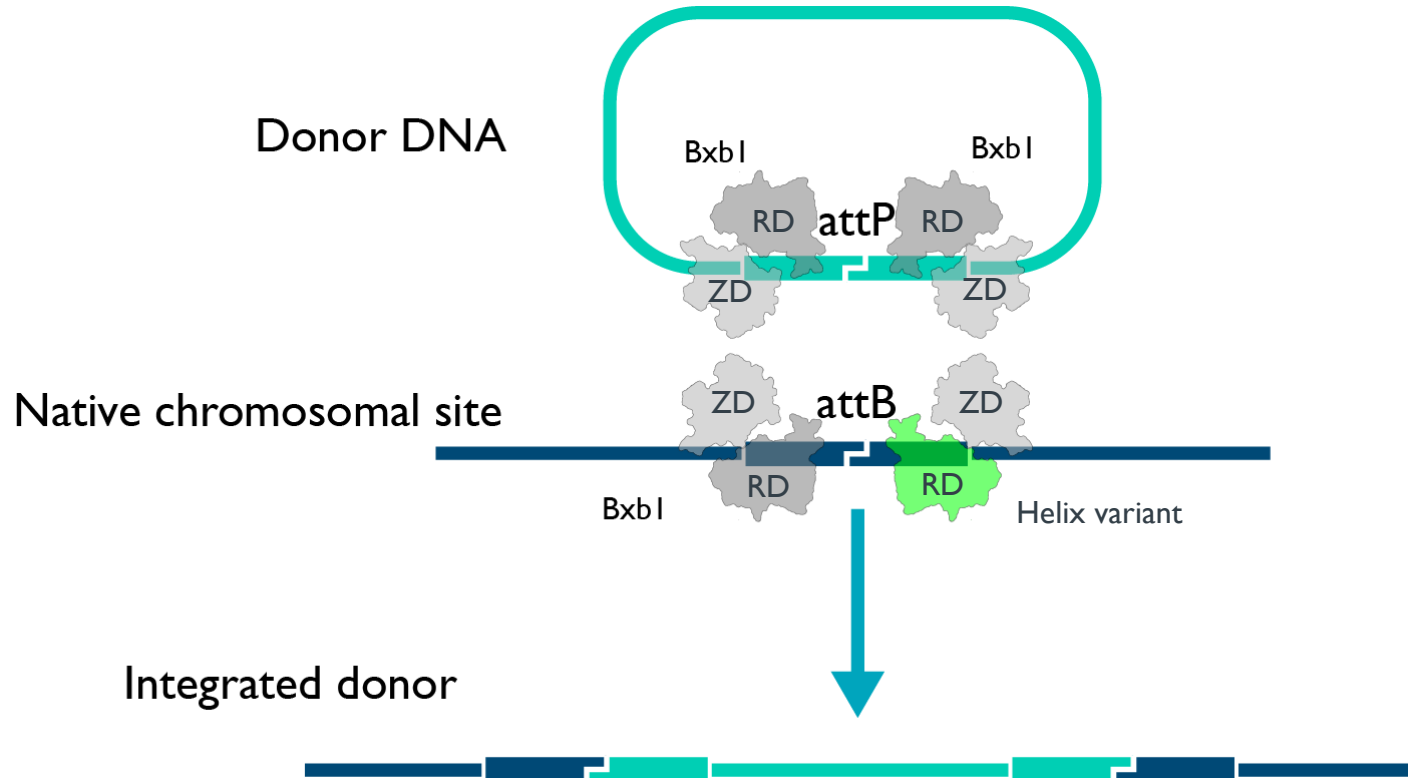
Frieder Fauser



Nicola Schmidt



# Selected Bxb1 Helices Enable Integration into the Genome of Human Cells

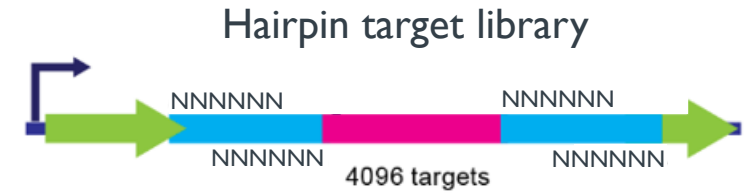
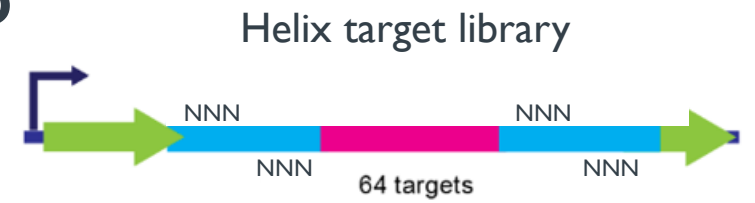
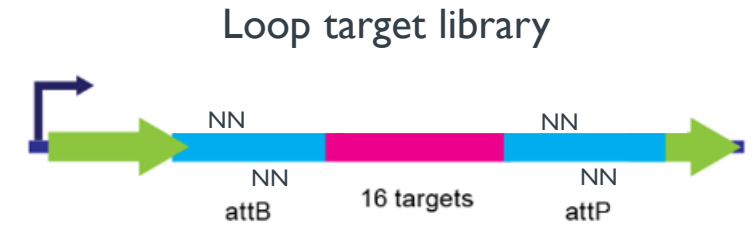
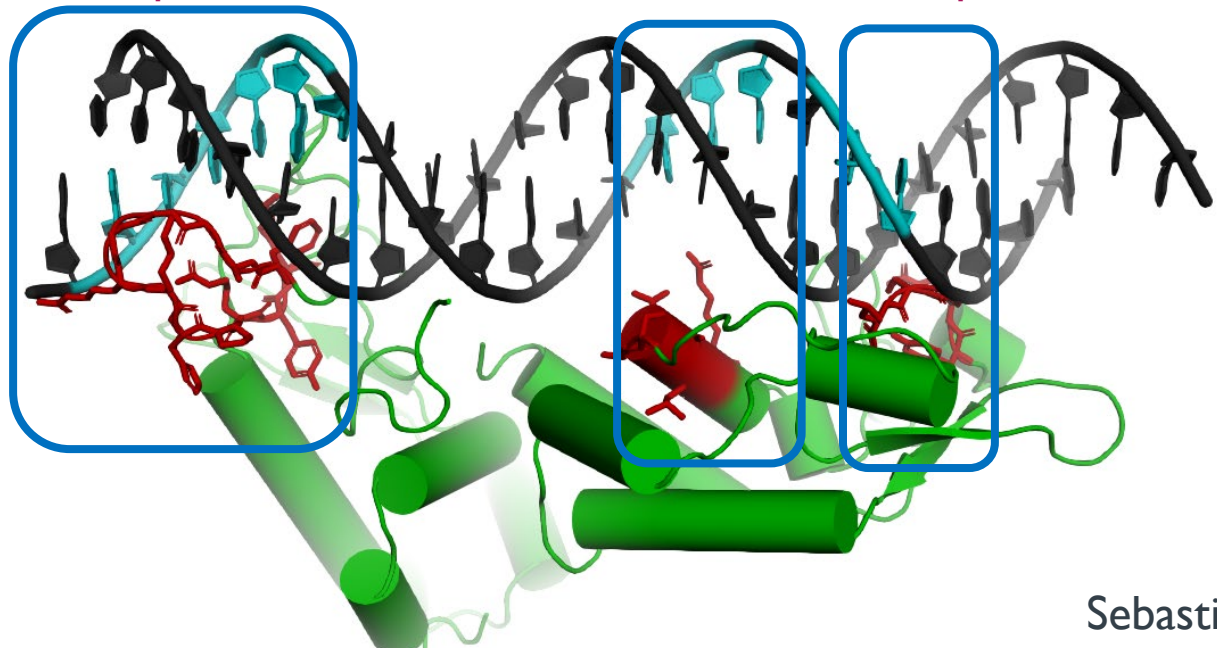
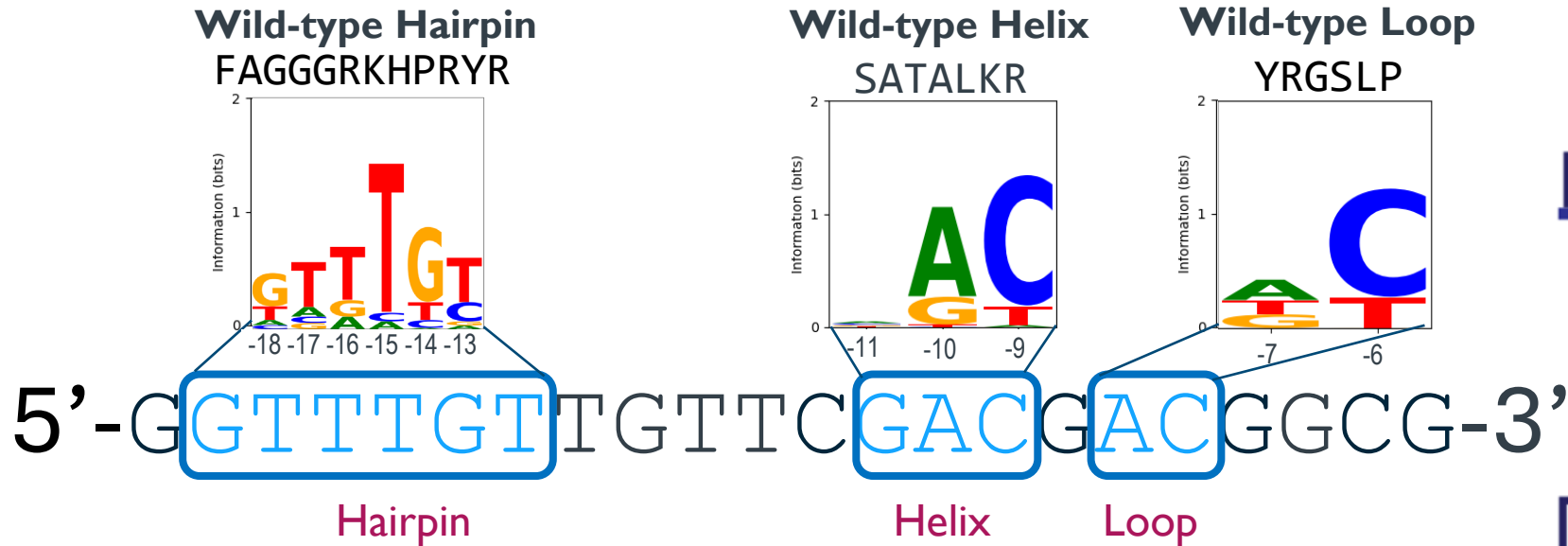


Frieder Fauser



Nicola Schmidt

# Specificity Characterization Using Random DNA Libraries in Bacteria



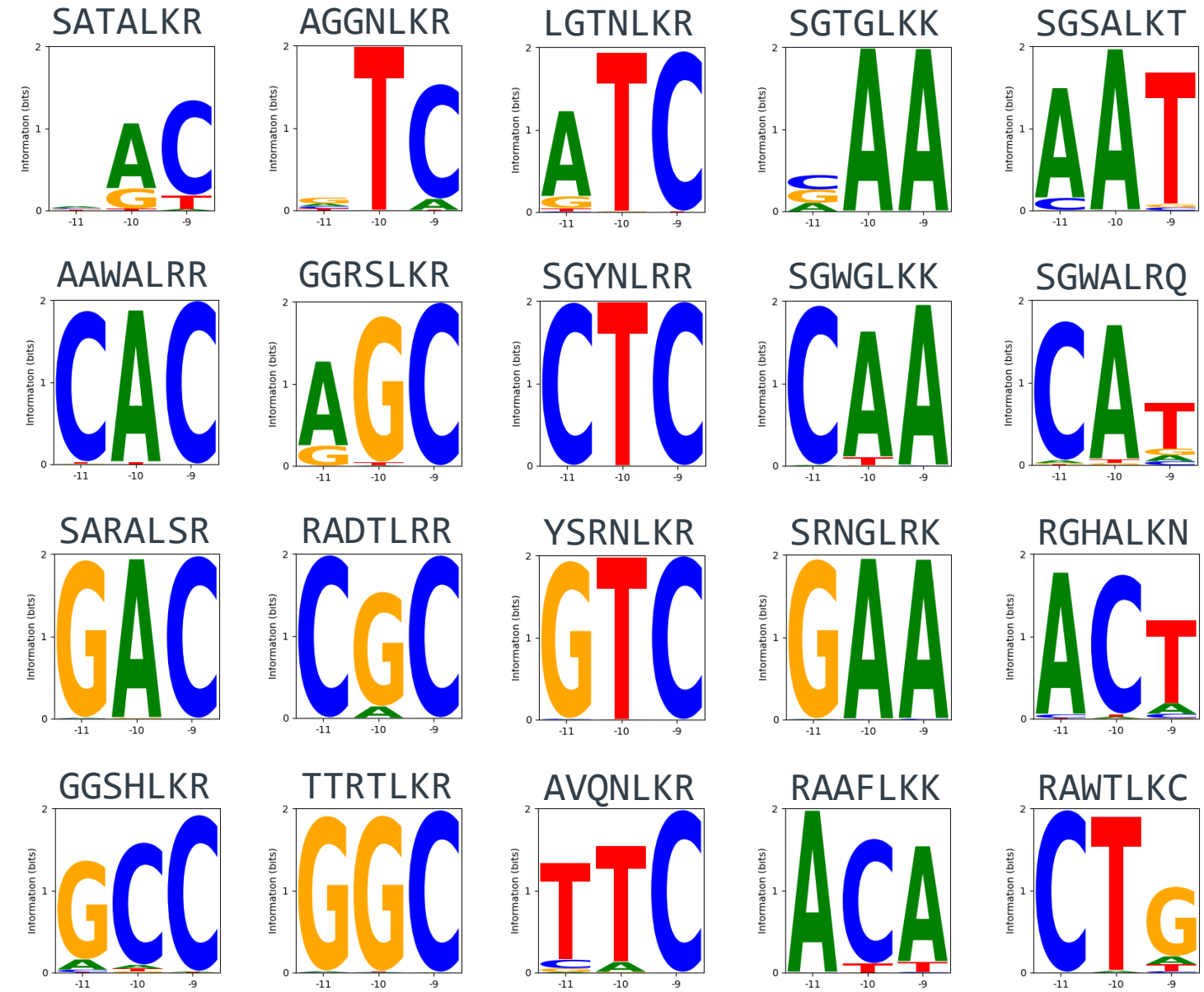
Recombined targets  
sequenced by NGS





# We Have Systematically Reprogrammed the Bxb1 Helix

Wild type Helix



64 helix selections against different DNA targets

Sebastian Arangundy

Luis Rodriguez





# Patterns for Helix vs. DNA Target Resemble Zinc Finger-DNA Interactions

Zinc Finger Examples

Wild type Helix



Zinc finger target normally shown as 5'-GCA-3'



Zinc finger target normally shown as 5'-TGA-3'

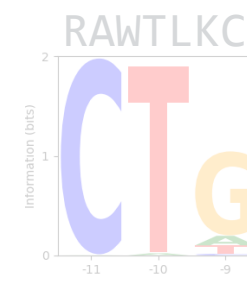
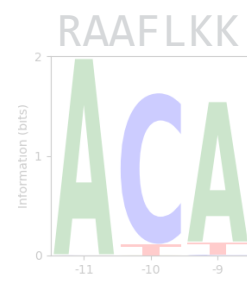
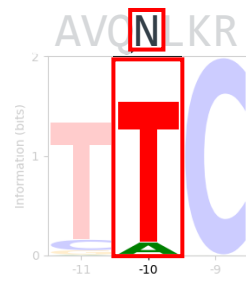
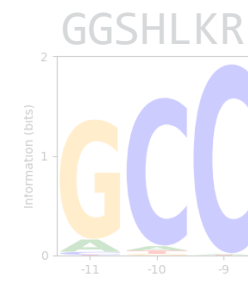
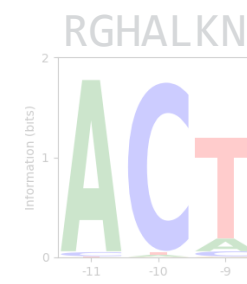
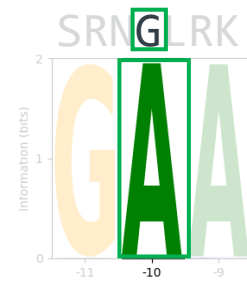
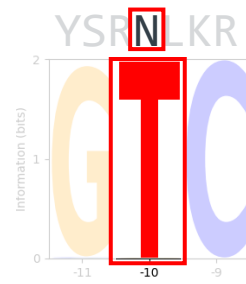
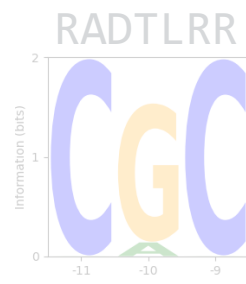
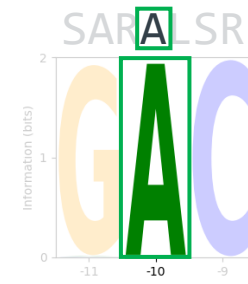
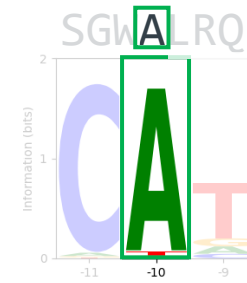
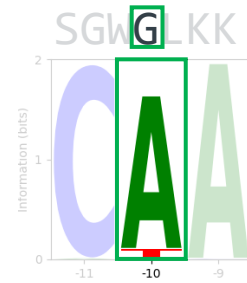
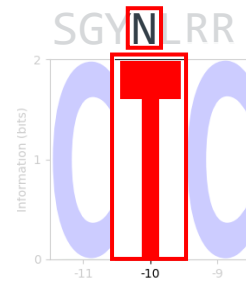
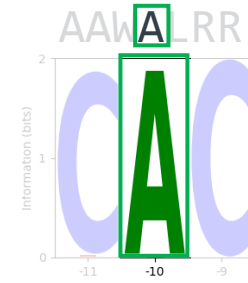
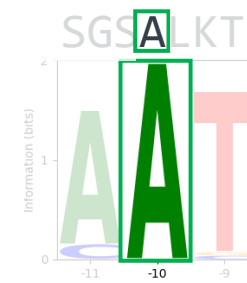
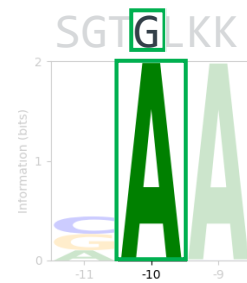
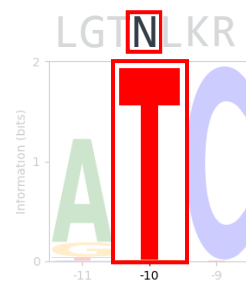
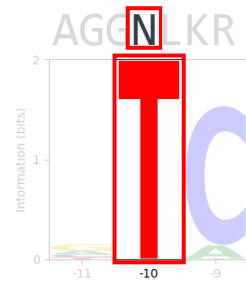
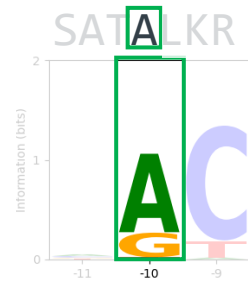
<sup>1</sup>Ichikawa et al. Nat. Biotech. 2023



# Patterns for Helix vs. DNA Target Resemble Zinc Finger-DNA Interactions

## Zinc Finger Examples

QSSN<sup>1</sup>LRT<sup>1</sup>  
 5'-T<sup>1</sup>T<sup>1</sup>  
 A A A-5'



DPSALIR<sup>1</sup>  
 5'-G<sup>1</sup>A<sup>1</sup>T<sup>1</sup>  
 C T A-5'

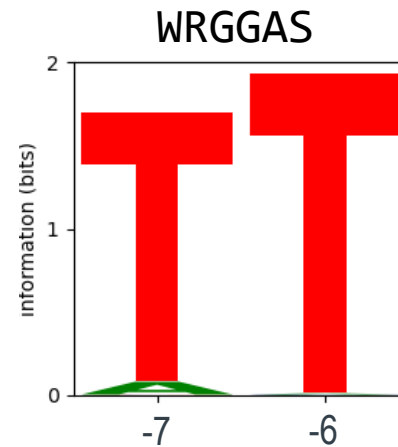
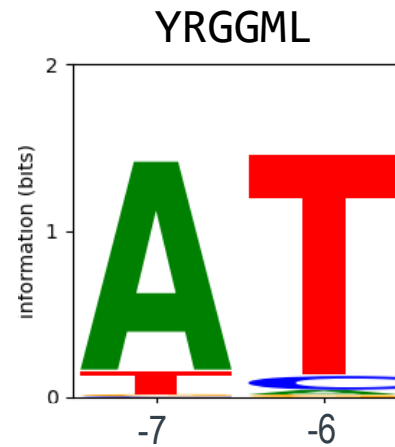
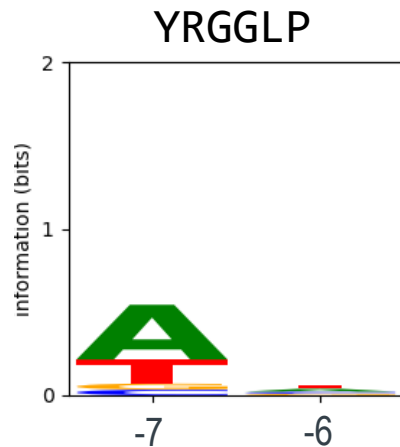
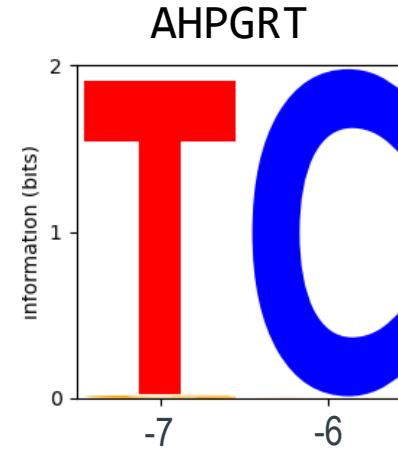
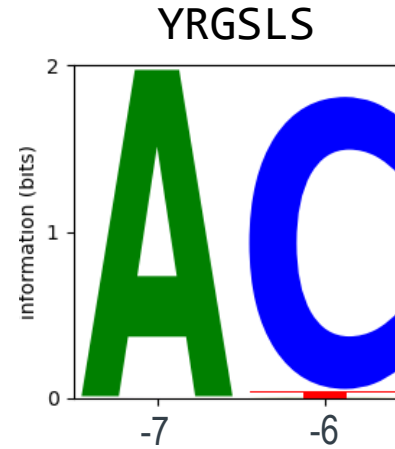
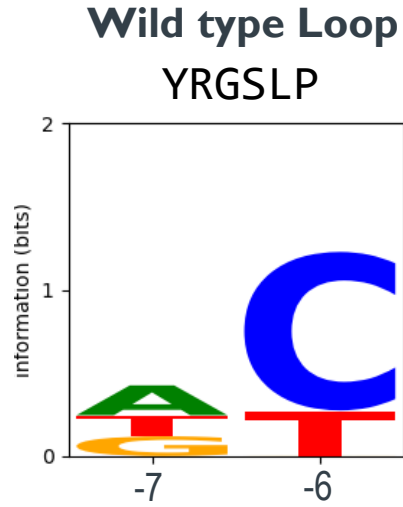
QHTGLNQ<sup>2</sup>  
 5'-T<sup>2</sup>A<sup>2</sup>T<sup>2</sup>  
 A T A-5'

<sup>1</sup>Ichikawa *et al.*  
 Nat. Biotech. 2023

<sup>2</sup>Wolfe *et al.*  
 Structure 2001



# Loop Specificity Can be Reprogrammed



16 loop selections against different DNA targets

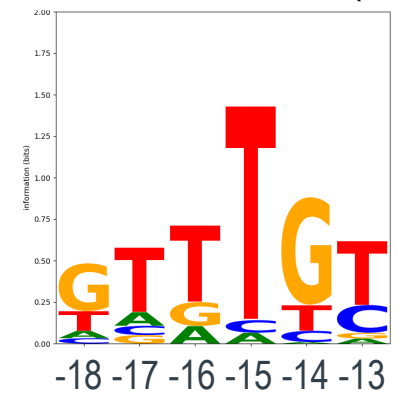
Sebastian Arangundy

Luis Rodriguez

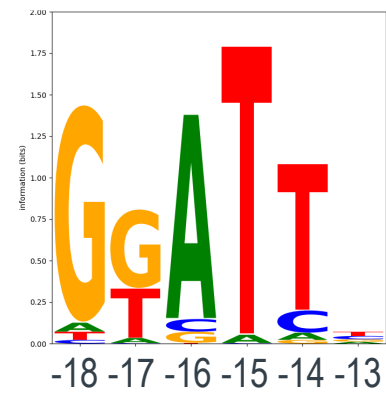


# Hairpin Specificity can be Reprogrammed

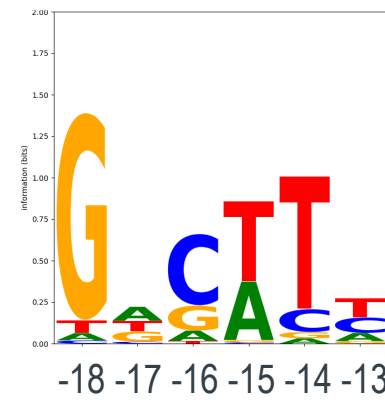
FAGGGRKHPRYR (wt)



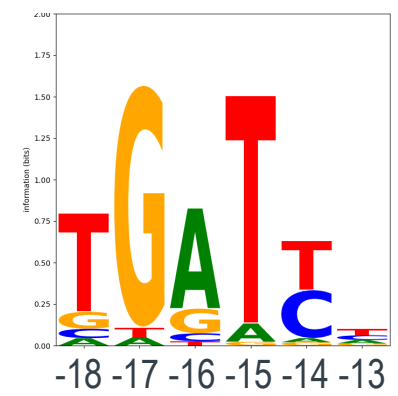
LARGSRKLALYR



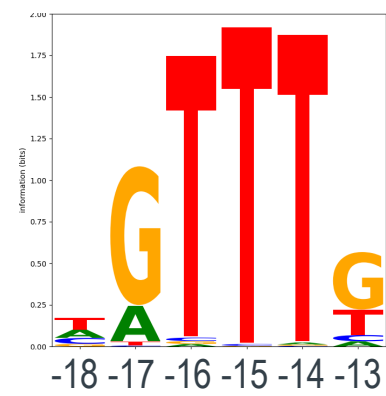
NARGNRKRGRYR



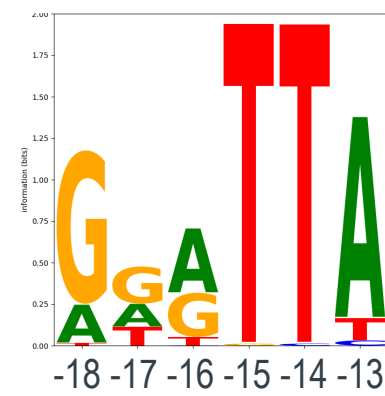
LARGRRKWARYR



MASGSRKTAIYY



SARGSRKLRVYR



> 100 different hairpin selections against different DNA targets

Sebastian Arangundy  
Luis Rodriguez





# Reprogrammed Bxb1 Submotifs can be Combined to Target Desired Sites

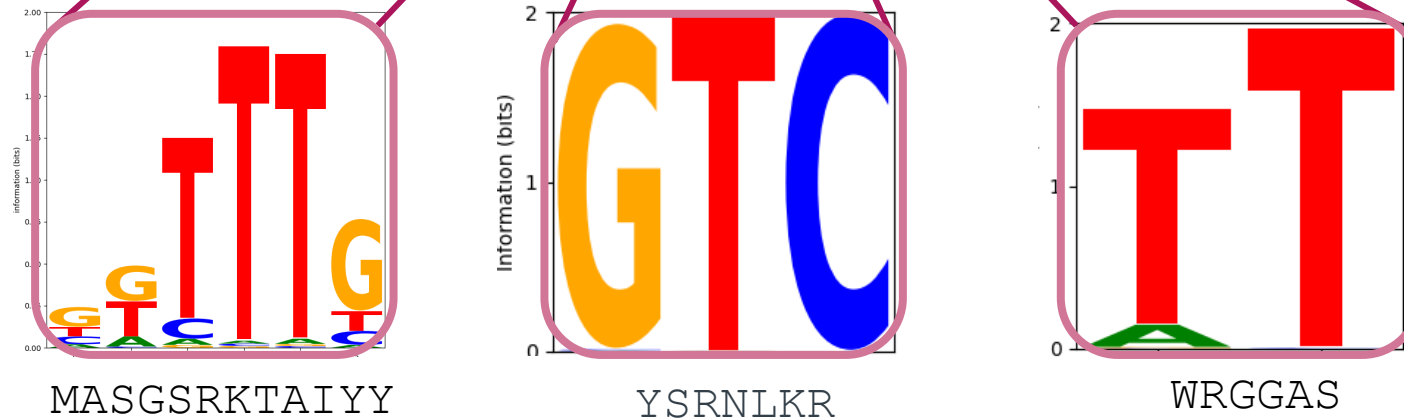
*Natural target half-site*

**GGCTTGTCGACGACGGCG**

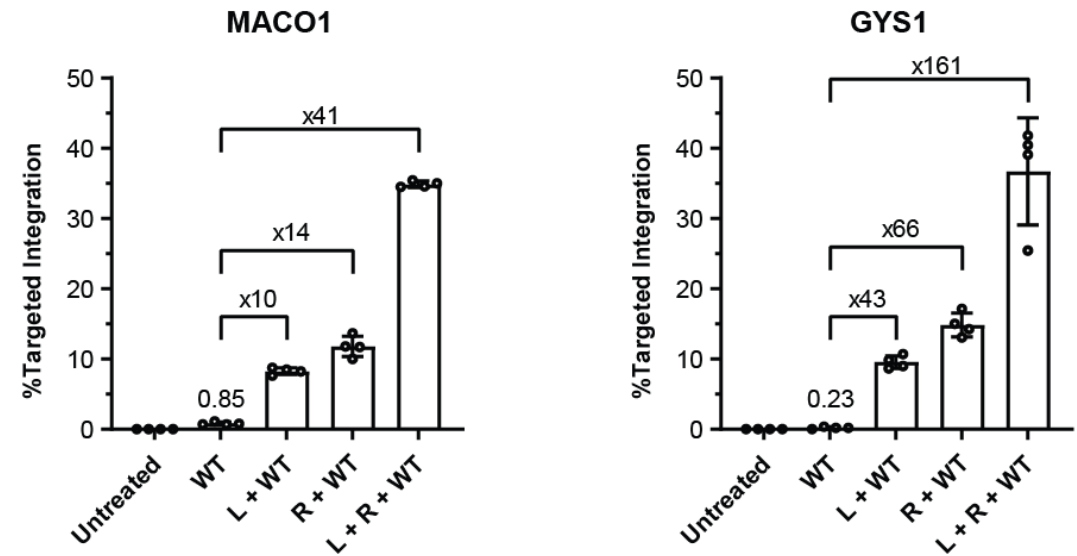
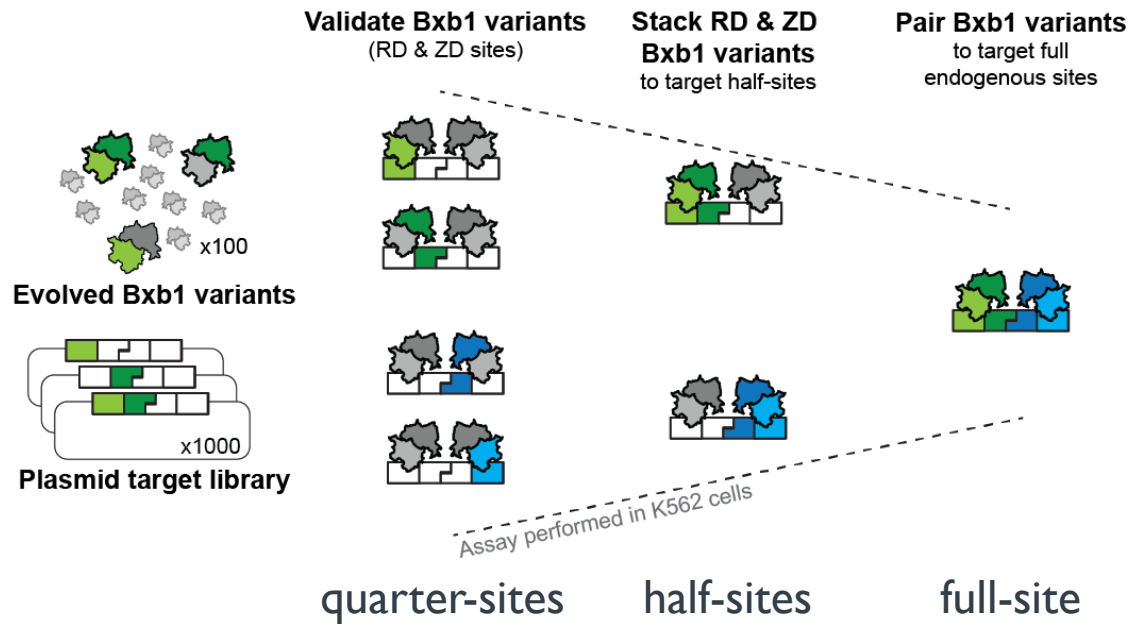
*Human safe-harbor locus half-site*

**G**GGTTT**G**AGTC****CCTT**GGCA**

*Engineered submotifs with novel DNA specificity*



# Strategy for Systematic Reprogramming Bxb1 to Target Human Sites

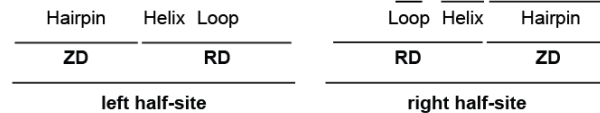


## Pseudo attB site: MACO1

5' -GCCCTTCTCCTACAGAGCAAGCAGCAGGGTAAATTCT  
CGGGGAAGAGGATGTCTCGTTCGTCCGTCCTTAAGA-5'

## Pseudo attB site: GYS1

5' -GGGATTCCTAACCCTGCACTCAGCTGCGGGAAGGCA  
CCCTAAGGGTATTGGCACGTGAGTCGACGCCCTCCGT-5'



## Bxb1 peptide sequences derived from directed evolution

	Helix	Hairpin
Wild-type Bxb1	SATALKR	FAGGGRKHPRYR
MACO1-L	SATALKR	MAGGHRKQALYR
MACO1-R	RAWSLKR	MAGGPRKKGRYR
GYS1-L	HGWSLKV	LARGSRKLALYR
GYS1-R	HGCTLKR	NARGNRKRGRYR

(Loop is wild-type)



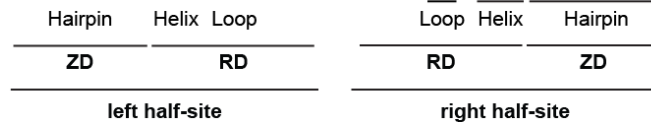
# This Strategy Works at Therapeutically Relevant Sites within the Human Genome

## TRAC: attB site

5' - TGGTGTCCAGGAGCCGAGGTATCGGTCCTGCCAGGGCC  
ACCACAGGTCTCGGCTCCATAGCCAGGACGGTCCCGG-5'

## AAVS1: attB site

5' - CTGAGCGCCTCTCCTGGGCTTGCCAAGGACTCAAACCC  
GACTCGCGGAGAGGACCCGAACGGTTCCTGAGTTTGGG-5'

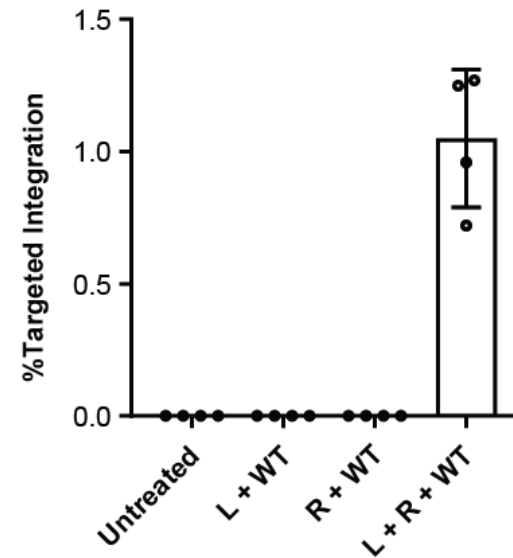


	Bxb1 peptide sequence from directed evolution		
	Loop	Helix	Hairpin
Wild-type Bxb1	YRGSLP	SATALKR	FAGGGRKHPRYR
TRAC-L*	YRGGLP	YGSALKQ	LARGPRKRAGYK
TRAC-R*	YRGGLP	SQWALKC	RAWGKRKYAYYQ
AAVS1-L	YRGGLP	YPWSLRR	KAWGSRKTRLYR
AAVS1-R	YRGGLP	AGGNLKR	MARGGRKSAIYY

\*TRAC-L and TRAC-R variants include an additional D257K mutation

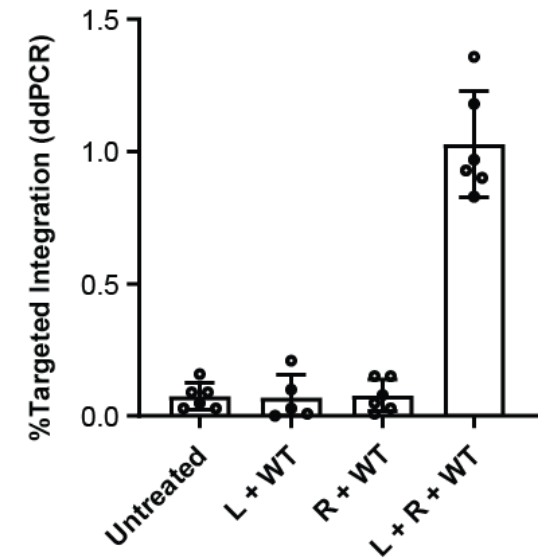
Frieder Fauser

## TRAC



Rakshaa Mureli

## AAVS1



Lifeng Liu

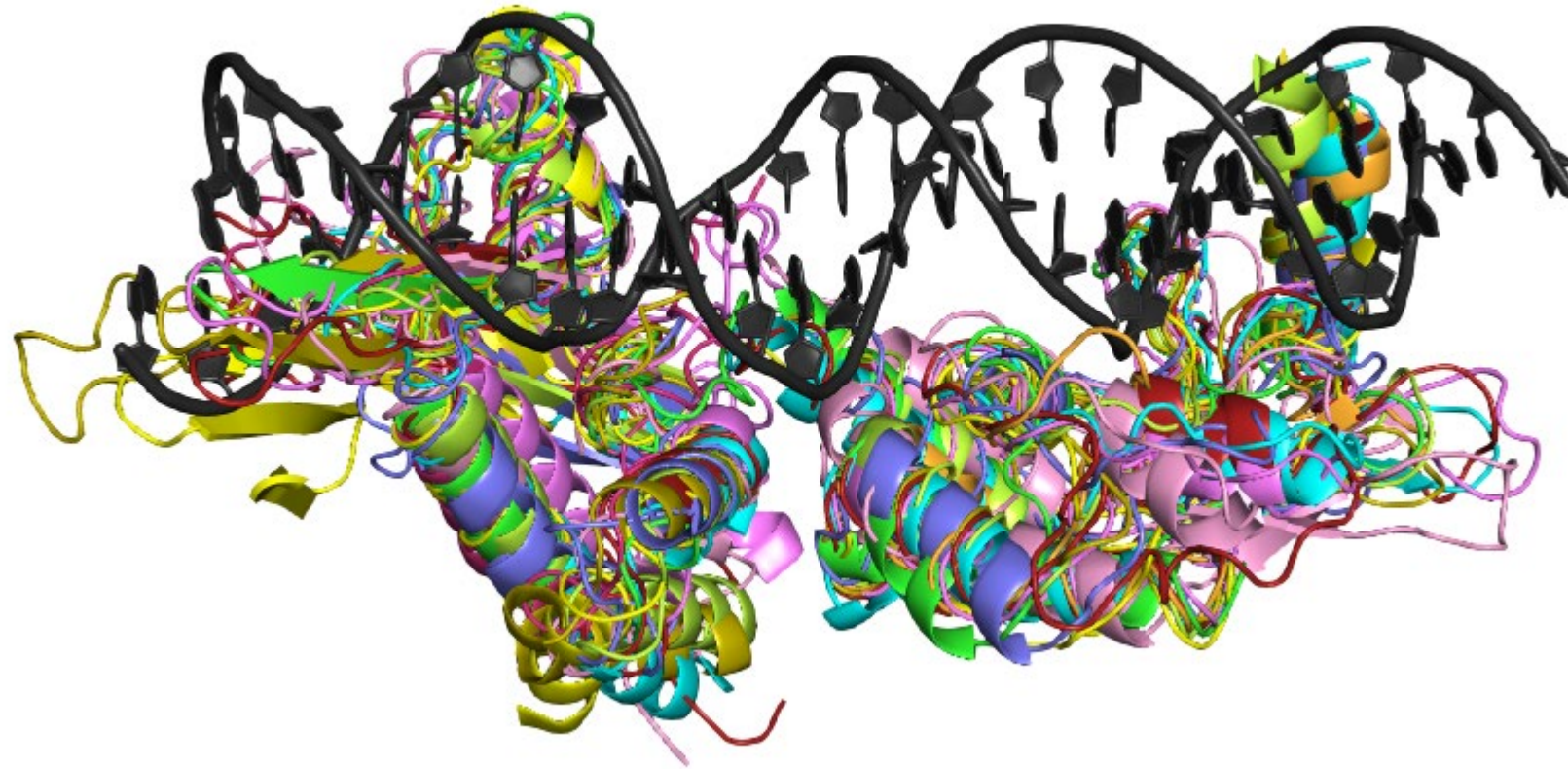


Nga Nguyen



# This Strategy Should Work with a Wide Variety of Natural Integrases

- Bxb1
- Pa01
- Kp03
- Nm60
- Si74
- BcyInt
- SscInt



Durrant et al. Nat Biotech (2022)  
Yarnall et al. Nat Biotech (2023)



## Summary

- ✔ Demonstrated serine integrase reprogramming for the first time
- ✔ The MINT platform enables insertion of large DNA cargo into the human genome
- ✔ The MINT platform should unlock new ways to treat genetic diseases
- ✔ Reprogramming strategy will likely apply to other integrases



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